

## Serological Survey of Newcastle Disease in Domestic Chickens in Sulaimani Province



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### Abstract

Newcastle disease is one of the serious diseases of poultry. It causes a great loss in poultry industry and domestic village chickens. Since domestic chickens in villages are free ranging for food, the chance of contact with wild birds which may act as a reservoir for many viral diseases is high.

The ELISA and Haemagglutination Inhibition (HI) test were used for the detection of antibodies against Newcastle disease virus in 500 serum samples collected from chickens with no history of vaccination from five regions in Sulaimani province during September and October 2006. Forty six percent of sera were positive by ELISA and 34.4% by HI test. Chi-square and t-tests were used for statistical analysis. A good correlation was observed between ELISA and HI titers (Correlation Coefficient=0.705205, T=3.80732) and there was a statistically significant differences between ELISA and HI test at 95% confidence level ( $P < 0.05$ ).

It was found that 46% of the domestic village chickens have protective antibody and 53.9% of chickens are susceptible to Newcastle disease virus infection.

**Keywords:** Newcastle disease, ELISA, HI, Sulaimni province.

### Introduction:

Newcastle disease virus is an avian paramyxovirus that produces pneumoencephalitis in young chickens, turkeys and other domesticated and wild birds characterized by respiratory symptoms, neurological symptoms, enteritis, haemorrhagic lesions and often with high mortality. In human, it may produce inflammation of the conjunctiva. Infection in humans is an occupational disease limited to workers handling infected birds [1,2]. The disease generally considered the most serious poultry disease throughout the world [3].

Newcastle disease causes up to 100% mortality in susceptible chickens. It is worldwide in distribution, while many species may be infected; dramatic losses are seen most often in domestic fowls and to

the lesser extent in turkeys and pheasants [4, 5].

The major constraint to production of village chickens in many developing countries is Newcastle disease [6, 7]. In these countries the circulating strains of NDV are capable of causing 100% mortality in unprotected birds [8].

Both epizootic and enzootic ND is recognized in village chickens. All village chickens are sold as live birds, for consumption or for breeding. It does not seem feasible to control this aspect of the epidemiology of the disease. Suitable vaccines seem to be the only answer for the control of NDV in village chickens [9-12].

People of villages in Sulaimani province from the past, have a history of rearing and breeding chickens and other birds for the purpose of meat and egg production, and even as a hobby. In spite of

the development of poultry production, many diseases are prevalent in Sulaimani province including ND which is the most serious disease and causes a great loss in poultry production which is a threat for the future rearing of poultry industry [13].

In the absence of vaccination and the presence of specific antibodies against NDV indicate that the birds have been infected by the virus at sometimes, but not necessarily that it was suffering from the disease at the time of sampling. In practice, a high antibody titer is indicative of a recent infection [14].

Newcastle disease virus may be employed as an antigen in a wide variety of serological tests, enabling neutralization or ELISA or HI tests to be used for diagnosis [15].

Several ELISA kits are available commercially for the detection of antibodies against NDV, and the main advantage of ELISA over more conventional tests, such as HI test, is that they can be semi-automated, enable results to be obtained rapidly, especially when sera are to be screened for antibodies against several viruses [16]. ELISA for detection of NDV antibodies shows high reproducibility, with high comparative sensitivity and specificity and correlate well with HI test [17,18].

#### **Materials and Methods:**

**Study groups:** Five hundred chickens were selected randomly throughout September and October 2006, from Sulaimani province which include 11 villages, with no history of clinical illness, and the chickens were apparently healthy with no history of vaccination. All chickens were divided into 5 groups according to their ages.

**Blood collection:** 1-2 ml blood were collected from brachial vein, allowed to clot at room temperature then centrifuged at

2500 rpm for 15 minutes, and then the sera were collected, labeled and stored at  $-20^{\circ}\text{C}$  for further analysis.

**Serological assay:**

**ELISA:** The sera were screened with a commercial, highly sensitive blocking-ELISA (SVANOVIR Newcastle Disease Abs ELISA Test Kit, SVANOVA, and UPPSALA, SWEDEN) to achieve a high negative predictive value. The procedure was followed according to the manufacturer's directions.

The percent inhibition (PI) value was used for interpretation of results.  $\text{PI} > 40$  were considered positive,  $\text{PI} = 30-40$  considered doubtful and  $\text{PI} < 30$  considered negative.

**Haemagglutination Inhibition Test:** The HI test was performed according to the Manual of standards for diagnostic Tests and Vaccination [19]. A titer greater or equal to  $4 \text{ Log}_2$  was considered positive.

**Statistical analysis:** Chi-square was used for statistical analysis of the prevalence of antibodies in different age groups and regions, and t-test was used for comparative analysis and sensitivity between ELISA and HI tests [20].

#### **Results:**

Seroprevalence of anti-NDV antibody in unvaccinated village chickens in different age groups:

A higher rate of antibodies (46%) was observed among chickens using blocking-ELISA than HI test (34.4%). A good correlation was obtained between ELISA (PI-value) and HI titres ( $\text{Log}_2$ ) (Correlation Coefficient=0.705205,  $T=3.80732$ ), there were a statistically significant relationship between ELISA and HI test at 95% confidence level ( $P < 0.05$ ). The highest prevalence rates of antibodies was detected in age group  $> 12$  months

(71.4%), followed by age group 9-12 months (54%), and the lowest (29%) was among age group 1-3 months by ELISA. The prevalence rates by HI test were 53.1%, 37.3% and 20.8% respectively. These

findings are shown in Table 1&2. There was a significant difference in the prevalence rates among different age groups by ELISA and HI test ( $P < 0.05$ ). .

**Table 1: Sero-prevalence of NDV antibody in unvaccinated chickens according to the age (ELISA test).**

Age	1-3 months	3-6 months	6-9 months	9-12 months	>12 months	Totals
Number of samples	93	104	78	100	105	480
Number of positive results (%)	27(29%)	39(37.5%)	26(33.3%)	54(54%)	75(71.4%)	221(46%)

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**Table 2: Sero-prevalence of NDV antibody in unvaccinated chickens according to the age (HI test).**

Age	1-3 months	3-6 months	6-9 months	9-12 months	>12 months	Totals
Number of samples	96	108	78	107	111	500
Number of positive results (%)	20(20.8%)	31(28.7%)	22(28.2%)	40(37.3%)	59(53.1%)	172(34.4%)

**Table 3: Sero-prevalence of NDV antibody in unvaccinated chickens according to the region (ELISA test).**

Region	Bakrajo	Chwarta	Zrgwez	Bazian	Penjwen	Totals
Number of samples	75	48	55	175	127	480
Number of positive results (%)	29(38.6%)	28(58.3%)	13(23.6%)	81(46.2%)	70(55.1%)	221(46%)

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**Table 4: Sero-prevalence of NDV antibody in unvaccinated chickens according to the region (HI test).**

Region	Bakrajo	Chwarta	Zrgwez	Bazian	Penjwen	Totals
Number of samples	78	49	57	183	133	500
Number of positive results (%)	22(28.2%)	21(42.8%)	10(17.5%)	65(35.5%)	54(40.6%)	172(34.4%)

Seroprevalence of anti-NDV antibody in chickens of different villages:

The highest prevalence rates of antibody were detected in Chwarta (58.3%) and Penjwen (55%) by ELISA, and the lowest rate (23.6%) in Zrgwes region. On the other hand, using HI test, the highest rates again was detected in Chwarta (42.8%) and Penjwen (40.6%), and the lowest in Zrgwez (17.5%). These findings are shown in Tables 3&4.

### Discussion:

A specific monoclonal blocking ELISA with ability to test sera from avian species for NDV-specific antibodies in a single dilution was used [21,22], the determination of a suitable cutoff point in ELISA and other quantitative serodiagnostic tests become a useful tool of analysis for better test performance, for reliable sensitivity and specificity.

The HI test is still the most widely used assay that requires cheap reagents, easy interpretation and it is a conventional serological method for measuring anti-NDV antibody levels in poultry sera and considered the standard laboratory method for diagnosis of NDV [23]. The prevalence of antibody titers by ELISA was higher than by HI test, with a mean antibody titer equal to 3 Log<sub>2</sub>. The Log<sub>2</sub> titers ranged from 0 to 10 in this study, the wider range of anti-NDV titers in unvaccinated chickens may be due to natural infection which is known to produce higher antibody titers than with vaccination [24]. This result was comparable to a study among unvaccinated chickens in Viet Nam in which the Log<sub>2</sub> titer ranged from 0 to 11 [25].

Studies in Middle East and other parts of the world have revealed comparable, lower

or higher prevalence of anti-NDV, 28.4% in Viet Nam [26], 43.6% in Ethiopia [27], 35% in Morocco [28], 69.5% in Iran [29], and 72% in Nigeria [30].

The variable environmental factors including climatic factors have an effect on dissemination of NDV, the incubation period was shortest at warmer temperature and the mortality rate was the highest [30].

In this study the results showed that anti-NDV was present in all age groups (grower and adults). Although, all groups are susceptible, it seems that by increasing age the seroprevalence increased and reaches the peak at > 12 months age group. Higher antibody titers in old age groups indicates more frequent exposure to the field virus which might have survived the disease at an earlier age and the mean HI titers increased with increasing age, again this wider range of antibody titers is an indication of natural infection which is known to produce high titers than vaccination [31]. Our findings were in agreement with other studies in Thailand [32], Viet Nam [26], and Bangladesh [33].

Anti-NDV antibody was found in chickens from all regions. There was a significant differences in antibody prevalence according to different regions ( $X=12.39$ ,  $P<0.05$ ), which was the lowest in Zrgwes and the highest in Chwarta and Penjwen. These two regions are located close to the border of Iran in which a high prevalence of anti-NDV was recorded [28]. Chwarta and Penjwen villages have a high density of chicken population (chicken flocks), this may lead to spread of the virus rapidly among chicken population. Similar observations were described elsewhere [34, 35].

The perpetuation of the virus in village poultry possesses a potential source

of the disease for the modern poultry sector in Kurdistan. The different geographical and climatic situation may have little to do with the epidemiology of ND in domestic village chickens. For the protection of susceptible chickens a routine vaccination program especially in spring and summer was suggested [29].

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## تۆیژینه وهیهکی سیرۆلۆجی ده‌بارهی راده‌ی بلا‌وبونه‌وه‌ی توش بون به‌ ڤایرۆسی نه‌خۆشی نیوکاسل

### له‌ نیو مریشکی خۆمائی له‌ گونده‌کانی پارێزگای سلیمانی

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### پوخته

نه‌خۆشی نیوکاسل به‌یه‌کیك له‌ نه‌خۆشیه‌ گرنه‌گه‌کانی په‌ له‌ وه‌ر ده‌ژمپردرپت که ده‌بیتنه‌ هۆی زیان گه‌یاندن به‌ پیشه‌سازی په‌ له‌ وه‌رو هه‌روه‌ها له‌ ناوبردنی ژماره‌یه‌کی زۆر له‌و مریشکانه‌ی که له‌ گونده‌کاندا به‌ خێو ده‌کرین. مریشکی کوردی له‌ گونده‌کاندا سه‌ریه‌ستانه‌ خۆراک ده‌خۆن و زیاتر په‌ یوه‌ندیان له‌ گه‌ل باندنه‌ کیویه‌کاندا هه‌یه‌ که به‌ سه‌رچاوه‌ی ڤایرۆسی نیوکاسل داده‌نرین و له‌ به‌رنه‌وه‌ وای ئی دی که مریشکه‌ مایه‌کانیش وه‌ک سه‌رچاوه‌ی نه‌خۆشیه‌که‌ ڤۆلیان هه‌بی.

به‌ مه‌به‌ستی ده‌ستنیشانکردنی ڤۆلی مریشکی مائی له‌ بلا‌وبونه‌وه‌ی نه‌خۆشی نیوکاسل له‌ گونده‌کاندا که سه‌ر به‌ پارێزگای سلیمانی نزیکی ۵۰۰ نمونه‌ی خۆین کۆکرایه‌وه‌ له‌و مریشکانه‌ی که به‌ هه‌ج شپۆه‌یه‌ک نه‌کوئراون به‌ ڤاکسینی نیوکاسل له‌ پینج ناوچه‌دا له‌ ماوه‌ی مانگی که‌ لاویژو ره‌زه‌ری سانی ۲۷۰۶ .

هه‌ردوو پشکنینی " HI, ELISA " به‌کاره‌ینرا بۆ ده‌ستنیشانکردنی دژه‌ ته‌نه‌کانی که دژ به‌ ڤایرۆسی نه‌خۆشی نیوکاسل هه‌یه‌و هه‌ردوو ڤیگه‌ی ( Chi-square , t-test ) به‌کاره‌ینرا بۆ ئیکدانه‌وه‌ ئاماریه‌کان.

ئەنە نجامدا ۴۶٪ پۈزەتتەپ بوو بە ھۆى پشكىنىنى "ELISA" و ۳۴،۴٪ پۈزەتتەپ بوو بە ھۆى پشكىنىنى "HI" و ئە نە نجامى ئەمەشدا پەيۈەندىيەكى باش دەسكەوت ئە نىۋان "PI value" ELISA ئەگەل (log<sub>2</sub>) HI titres . ئە رووى نامارەوہ پەيۈەندىيەكى پربايەخ دەست كەوت ئە نىۋان HI, ELISA ئە ۹۵٪ ئە ناستى (P<۰،۰۵) .  
ھەرۈەك دەرئە نجامى تۈۈزۈنەوہكان دەريانخست كە ۴۶٪ ئە مريشكە مائىيەكانى گۈندەكان دژە تەنى پاريزەريان ھەيە كە پاريزەگاريان ئى دەكات دژى نەخۈشى نيوكاسل و ۵۳،۹٪ ئە مريشكەكان ھەستيارن بۇ تووشبون بە نەخۈشى نيوكاسل ، ئەبەرئەوہ پروسەى كوتان بە بەردەوامى يۈۈستە بۇ مريشكە مائىيەكان ئە گۈندەكاندا ئە دژى نەخۈشى نيوكاسل .

### مسح مصلي لخمج فيروس مرض النيوكاسل في الدجاج المحلي لقرى محافظة السليمانية

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### الخلاصة

مرض النيوكاسل أحد الأمراض المهمة في الدواجن , غالباً ما يسبب خسائر كبيرة في صناعة الدواجن وكذلك في تربية الدواجن المحلية. لاسيما كون الدجاج المحلي المربي في القرى يكون عادة طليق التربية. وعليه فهو على تماس مع بقية الطيور البرية التي تعتبر حاملة للمرض أيضاً. ولعرقه وبائية مرض النيوكاسل في الدجاج المحلي المربي في قرى السليمانية , تم جمع ۵۰۰ عينة مصلية من دجاج غير ملقح أصلاً من خمسة مناطق من المحافظة خلال شهري أيلول وتشرين الأول لسنة ۲۰۰۶ .  
أستخدم فحصي الاليزا وتشبيط التلازن لاثبات وجود الأضداد لفيروس مرض النيوكاسل. كما أستخدم التحليلين الأحصائيين كاي-سكوير و تي- تيسست على المصول المجمع حيث ظهر بأنه ۴۶٪ و ۳۴،۴٪ من المصول كانت موجبة بفحص الاليزا و تشبيط التلازن على التوالي. كما لوحظ بأنه هنالك ترابط جيد بين فحص الاليزا (قيمة- PI) و الحجم المعياري (log<sub>2</sub>) لفحص تشبيط التلازن , كما ان هنالك علاقة احصائية معنوية بين فحص الاليزا وتشبيط التلازن عند ۹۵٪ بفرق معنوي (P< 0.05) . أظهرت النتائج بأن ۴۶٪ من الدجاج المحلي كان حاملاً لأضداد فيروس مرض النيوكاسل و ۵۳،۹٪ منه كان معرض للأصابة بالمرض و عليه يجب وضع خطة مبرمجة لتلقيح الدجاج المحلي في القرى ضد مرض النيوكاسل.